With Col. D. Bruce's Compliments,
Royal Army Medical College,
Millbank, S.W.

[*From the* PROCEEDINGS OF THE ROYAL SOCIETY, B, VOL. 83, 1911.]

3091

B

Experiments to Ascertain if Antelope may Act as a Reservoir of the Virus of Sleeping Sickness (Trypanosoma gambiense).

BY COLONEL SIR DAVID BRUCE, C.B., F.R.S., A.M.S.; AND CAPTAINS A. E. HAMERTON, D.S.O., AND H. R. BATEMAN, R.A.M.C.

WELLCOME INSTITUTE LIBRARY
Coll. welTROmec
Call No. pam WC705 1911 B88e

SLEEPING SICKNESS BUREAU LIBRARY.

SLEEPING SICKNESS BUREAU RECEIVED 9 - MAR 1911

22200130682

[*Reprinted from the* PROCEEDINGS OF THE ROYAL SOCIETY, B. Vol. 83]

Experiments to Ascertain if Antelope may Act as a Reservoir of the Virus of Sleeping Sickness (Trypanosoma gambiense).

By Colonel Sir DAVID BRUCE, C.B., F.R.S., A.M.S.; and Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, R.A.M.C. (Sleeping Sickness Commission of the Royal Society, Uganda, 1908–10).

(Received November 9, 1910,—Read January 19, 1911.)

LIBRARY.

Introduction.

The question of a reservoir of the virus of Sleeping Sickness, other than man and his domestic animals, is of the utmost importance.

Now man and the domestic animals have been removed from the Lake-shore of the mainland for some two and a half years, and from the islands since September, 1909. The effect of this depopulation has been to make a two mile area along the northern shores of the Lake virtually a game reserve, in which water-buck, bush-buck, reed-buck, Speke's *Tragelaphus*, hippopotami, wild pig, and other large game abound. The game water freely at the Lake-shore, and small herds of antelope may frequently be seen grazing on the grassy hillsides overlooking the Lake.

Notwithstanding the removal of man and his domestic animals, the Lake-shore *Glossina palpalis* continued to infect susceptible animals with Sleeping Sickness up to the end of March, 1910. The Commission, therefore, endeavoured to find answers to the following questions:—

1. Can antelope be infected with Sleeping Sickness by the bites of laboratory-bred and laboratory-infected *Glossina palpalis?*

2. If antelope can be infected with the virus of Sleeping Sickness, can they transmit the infection to laboratory-bred *Glossina palpalis* when these flies are allowed to feed upon them? Further, if these *Glossina palpalis* become infected, can they transmit the virus to susceptible animals?

3. If these *Glossina palpalis* become infected with the virus of Sleeping Sickness, what percentage are so infected?

4. How does Sleeping Sickness affect the health of the antelope?

5. Lastly, are antelope living in the fly-area naturally infected with Sleeping Sickness?

Should all these questions be answered in the affirmative, the importance of the observation is patent. The continued infectivity of the flies on the Lake-shore would be explained. Whilst the movements of man and the domestic animals can, to some extent, be controlled by administrative measures, the movements of antelope in search of new grazing grounds

would be almost impossible to check, owing to the difficult nature of the forested and elephant-grass country which in so many parts borders the Uganda shores of the Lake. Game laws would require modification, and the destruction of the game in the neighbourhood of the lakes and *palpalis*-frequented rivers would become a factor in the control of Sleeping Sickness.

To answer the above queries the following experiments were devised and carried out:—

1. *Can Antelope be Infected with Sleeping Sickness by the Bites of Laboratory-bred and Laboratory-infected Glossina palpalis?*

Eleven buck in all were employed in this experiment. Four bush-buck (*Tragelaphus sylvatica?*), six reed-buck (*Cervicapra arundinacea*), and one water-buck (*Cobus defassa*). Other buck, such as "oribi" (*Cephalophus grimmi*) and "entalaganya" (*Cephalophus equatorialis*), were obtained by the Commission, but did not survive long in captivity.

As each buck arrived its blood was usually injected, subcutaneously, into monkeys or rats, to ascertain if the blood was naturally infected with trypanosomes. The method then adopted in each case to infect the antelope with Sleeping Sickness was as follows: A cage of clean* laboratory-bred *Glossina palpalis* was fed on a monkey known to be infected with a *human strain* of *Trypanosoma gambiense*. This cage of artificially-infected flies was then fed on healthy animals until one of the animals (monkeys) became infected with Sleeping Sickness. Having proved that the flies were infective, the cage of flies was then fed, about five minutes daily, for several days, on one or other of the buck. The buck's blood was then examined daily for trypanosomes, and was further tested for Sleeping Sickness by its inoculation into monkeys or rats.

The experiments follow in full detail.

Experiment 2328. Bush-buck.

This bush-buck was fed on for five days (March 24, 25, 26, 28, and 29, 1910) by laboratory-bred *Glossina palpalis* which were known to be infected with a *human strain* of *Trypanosoma gambiense*.

On April 8, 15 days after the first feed of the infected flies on this buck, 5 c.c. of the buck's blood were injected, subcutaneously, into a healthy monkey.

April 19, monkey showed *Trypanosoma gambiense* in its blood. The trypanosomes were verified by examination in fresh and stained preparations of the blood.

Result.—Positive.

Remarks.—The preliminary inoculation of the blood into susceptible animals, to

* The word "clean," applied here and throughout this paper to laboratory-bred *Glossina palpalis*, means flies which have newly hatched out from pupæ in the laboratory and have never fed.

ascertain if the buck's blood naturally harboured trypanosomes, was not made in this case. Though examined almost daily from March 29 to August 5, 1910, *Trypanosoma gambiense* was never seen in the blood of this antelope. Fresh and stained blood films were made and examined. On one occasion only—April 20—*Trypanosoma ingens* was seen in a fresh preparation. Monkeys are not inoculable with this parasite.

Experiment 2357. Reed-buck.

April 8, 1910, 5 c.c. of this buck's blood were injected, subcutaneously, into a normal monkey, to ascertain if buck's blood naturally harboured trypanosomes. This monkey's blood was examined bi-weekly for a month. Monkey remained healthy, no trypanosomes appearing in its blood.

Buck was fed on for 12 days, between April 7 and 20, 1910, by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense.* On the 13th day after the first feed of these flies, 3 c.c. of the buck's blood were inoculated into a normal monkey. Examined bi-weekly for a month this monkey remained healthy.

Trypanosoma gambiense having appeared in the buck's blood on May 2, a further inoculation of 5 c.c. of its blood was made into a normal monkey on this date. This monkey showed *Trypanosoma gambiense* in its blood on May 10.

Result.—Positive.

Remarks.—This reed-buck was evidently free from any trypanosome infection inoculable into monkeys on its arrival at the laboratory. *Trypanosoma gambiense* appeared for the first time in the buck's blood in scanty numbers on May 2, 1910, 25 days after the first feed of the supposed infective *Glossina palpalis.* On May 3 and 4, the trypanosomes were fairly numerous in the blood, and on the 5th and 6th, scanty numbers only were seen. After May 6, *Trypanosoma gambiense* was never again seen in the blood, though examined for almost daily up to August 5, 1910. The long period of 25 days which elapsed between the first feed of the supposed infected *Glossina* and the appearance of the trypanosomes in the buck's blood may be accounted for by the supposition that the infected fly or flies in the cage which was fed on the buck from April 7 to April 19 had died before they had fed on the buck. On April 20, a fresh cage of *Glossina palpalis*, known to be infected with a *human strain* of *Trypanosoma gambiense* was fed. once only, on this buck, and on the 12th day after this feed, on May 2, trypanosomes appeared in the buck's blood. This supposition is probably correct, and further accounts for the failure of the inoculation on April 20.

Experiment 2359. Reed-buck.

On April 8, 1910, 5 c.c. of this buck's blood were injected, subcutaneously, into a normal monkey, to ascertain if buck's blood naturally harboured trypanosomes. This monkey's blood was examined bi-weekly for a month. Monkey remained healthy.

Buck was fed on for six days (April 25—30, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense.*

On May 6, the 11th day after flies' first feed, *Trypanosoma gambiense* appeared in buck's blood in fair numbers, and 5 minims were injected, subcutaneously, into normal white rat. On May 11 this rat showed *Trypanosoma gambiense* in its blood.

Result.—Positive.

Remarks.—This reed-buck was evidently free from any trypanosome infection inoculable into monkeys on its arrival at the laboratory. *Trypanosoma gambiense* appeared for the first time in its blood in fair numbers on May 6, 1910, again on 7th, not examined for on 8th, scanty on 9th, absent on 10th, scanty on 11th, very numerous on 12th, and never seen again, though examined for almost daily, till August 5.

Experiment 2371. Bush-buck.

On April 13, 1910, 5 c.c. of this buck's blood were injected, subcutaneously, into a normal monkey, to ascertain if buck's blood naturally harboured trypanosomes. This monkey's blood was examined bi-weekly for a month. Monkey remained healthy.

Buck was fed on for eight days (April 22 and 23, and from April 25 to 30, 1910, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense.*

On May 4, the 12th day after the infected flies' first feed on the buck, *Trypanosoma gambiense* appeared in the buck's blood in fair numbers.

On May 5, trypanosomes were numerous, and 2 c.c. of the buck's blood were injected, subcutaneously, into a normal monkey. On May 13 this monkey showed *Trypanosoma gambiense* in its blood.

Result.—Positive.

Remarks.—Buck was free from trypanosomes inoculable into a monkey on its arrival at the laboratory. *Trypanosoma gambiense* appeared in buck's blood for the first time, in scanty numbers, on May 3, 1910, and again in fair numbers on May 4 ; thereafter no trypanosomes were seen up to August 5, 1910, though the blood was almost daily examined.

Experiment 2378. Water-buck.

On April 13, 1910, 5 c.c. of this buck's blood were injected, subcutaneously, into a normal monkey, to ascertain if the buck naturally harboured trypanosomes in its blood. This monkey was examined bi-weekly for one month ; monkey remained healthy.

Buck was fed on for eight days (April 22, 23, 25 to 30, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense. Trypanosoma gambiense* was never seen in this buck's blood, though examined for almost daily from April 22 to August 5, 1910.

On May 5, 5 c.c. of the buck's blood were injected, subcutaneously, into a normal monkey, an interval of 13 days having elapsed since first feed of the infected flies. This monkey showed *Trypanosoma gambiense* in its blood on May 13, 1910.

Result.—Positive.

Remarks.—Buck was free from trypanosomes inoculable into a monkey on its arrival at the laboratory. Though *Trypanosoma gambiense* never appeared in the buck's blood, yet a positive result was obtained on its inoculation into a monkey, and, as will be seen below (Table II), clean laboratory-fed flies fed on this buck for several days became infected with *Trypanosoma gambiense.*

Experiment 2427. Reed-buck.

On May 4, 1910, 1 c.c. of this buck's blood was injected, subcutaneously, into a normal white rat. This rat, examined bi-weekly, for one month, remained healthy.

Buck was fed on for six days (May 2 to 7, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense.*

On May 9, the seventh day after infected flies' first feed on the buck, *Trypanosoma gambiense* appeared in scanty numbers in its blood for the first time. One cubic centimetre of the blood was then injected, subcutaneously, into a normal white rat. On May 16, *Trypanosoma gambiense* appeared in this rat's blood.

Result.—Positive.

Remarks.—On its arrival at the laboratory the buck was free from trypanosomes inoculable into a rat. A few *Trypanosoma gambiense* appeared for the first time on May 9, 1910, in buck's blood ; they were present in fair numbers on the 10th, scanty

again on the 11th, and were seen for the last time, in fair numbers, on May 12. Almost daily blood examinations were made, with negative results, up to August 5, 1910.

Experiment 2428. Bush-buck.

On May 4, 1910, 1 c.c. of this buck's blood was injected, subcutaneously, into a normal white rat. This rat remained healthy, its blood being examined for one month after the injection.

Buck was fed on for 13 days (May 2—16, inclusive, May 9 and 15 being excepted) by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense*. *Trypanosoma gambiense* was never seen in this animal's blood, though examined for almost daily from May 4 to August 5, 1910.

On May 16, 14 days after infected flies' first feed on the buck, a few drops of the buck's blood were injected, subcutaneously, into a normal white rat. This rat showed *Trypanosoma gambiense* in its blood on May 23. No trypanosomes were ever seen in buck's blood.

Result.—Positive.

Remarks.—On its arrival at the laboratory the buck was free from trypanosomes inoculable into a rat. No trypanosomes were ever seen in the buck's blood, nevertheless its blood was infective on inoculation, and, as will be seen later (Table II), capable of infecting clean laboratory-bred *Glossina palpalis*.

Experiment 2429. Reed-buck.

On April 13, 1910, 5 c.c. of this buck's blood were injected, subcutaneously, into a normal monkey. This monkey remained healthy, its blood being examined bi-weekly for a month after the injection. On May 3, 1910, 1 c.c. of the buck's blood was injected, subcutaneously, into a normal rat. This rat remained healthy, its blood also being examined bi-weekly for a month after the injection.

The buck was fed on for eight days (May 2—7, and 9 and 10, inclusive) by *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense*.

On May 11, the ninth day after the infected flies' first feed on the buck, *Trypanosoma gambiense* appeared in scanty numbers in its blood for the first time. On May 13, the trypanosomes being numerous in the blood of the buck, a few drops of the blood were injected, subcutaneously, into a normal white rat. This rat showed *Trypanosoma gambiense* in its blood on May 17.

On July 2, 1910, this buck accidentally broke its leg and had to be killed, 4 c.c. of its heart's blood being injected into a normal monkey. This monkey showed *Trypanosoma gambiense* in its blood on July 12.

Result.—Positive.

Remarks.—On its arrival at the laboratory the buck was free from trypanosomes inoculable into monkeys or rats. *Trypanosoma gambiense* appeared in its blood for three days —on May 11 for the first time in scanty numbers, on May 12, many, and on May 13, very many—thereafter no trypanosomes were seen, though almost daily examinations were made up to July 2, 1910. It will be noted that the buck's blood was still infected with *Trypanosoma gambiense* on July 2—that is, 50 days after the last date (May 13) that trypanosomes were seen in it.

Experiment 2431. Reed-buck.

On May 3, 1910, 1 c.c. of this buck's blood was injected, subcutaneously, into a normal white rat. Rat remained healthy, its blood being examined bi-weekly for a month after the injection.

The buck was fed on for six days (May 2—7, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense.*

On May 12, 1910, the tenth day after the infected flies' first feed, *Trypanosoma gambiense*, in scanty numbers, appeared for the first time in the blood of the buck. On May 13, the trypanosomes being numerous in the blood of the buck, a few drops of its blood were injected, subcutaneously, into a normal white rat. This rat showed numerous *Trypanosoma gambiense* in its blood on May 20.

Result.—Positive.

Remarks.—On its arrival at the laboratory the buck was free from trypanosomes inoculable into rats. *Trypanosoma gambiense* appeared in the buck's blood, in scanty numbers, for the first time on May 12, and were present in large numbers on May 13 and 14.

We have now to record one of the most important and suggestive observations in this series of experiments. *From May* 14 *to June* 27 *the blood was examined almost daily, and on the latter date—June* 27—44 *days after trypanosomes were last seen*, Trypanosoma gambiense *reappeared for one day in this buck's blood in fair numbers.* Their identity was established by careful examination in wet and stained preparations of the blood.

(N.B.—Two mechanical transmission experiments were carried out with this buck. In the first, 50 flies were used and were fed on the buck for the three days the trypanosomes were seen in its blood. *Result.*—Negative. In the second, 100 flies were used, and were fed for four days on the buck. No trypanosomes were seen in the buck's blood during these days. *Result.*—Negative. In the first experiment four hours, and in the second one hour, elapsed between the feed on the buck and the feed on the healthy monkey.)

Experiment 2445. Reed-buck.

No preliminary inoculation of this buck's blood was made on its arrival at the laboratory.

The buck was fed on for seven days (May 6 and 7, and 9—13, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense.*

On May 14, 1910, the eighth day after the infected flies' first feed, *Trypanosoma gambiense* appeared in the blood of the buck for the first time. On May 20 the buck died, and a small quantity of its heart's blood was injected, subcutaneously, into a normal white rat. On June 2 rat showed *Trypanosoma gambiense* in its blood. (This rat was overlooked from May 24 to June 2, and its blood was not examined between these dates.)

Result.—Positive.

Remarks.—The blood of this buck was (with the exception of May 8) examined daily from May 6 to 13 with negative results for trypanosomes. On May 14, *Trypanosoma gambiense* appeared for the first time in its blood in scanty numbers ; on the 16th, 17th, and 18th they were very numerous ; on the 19th they were again scanty, and on the day of death, May 20, 1910, no trypanosomes could be found, the peripheral and heart's blood being examined.

Table I gives results of feeding infected *Glossina palpalis* on healthy antelope.

Table I.

No. of experiment.	Species of antelope.	No. of days infected flies fed.	No. of days before trypanosomes appeared.	Result.		Remarks.
				Positive.	Negative.	
2328	Bush-buck	5	—	+		Trypanosomes never seen.
2357	Reed-buck	12	25	+		
2359	„	6	11	+		
2371	Bush-buck	8	12	+		
2372	„	6	8	+		
2378	Water-buck	8	—	+		Trypanosomes never seen.
2427	Reed-buck	6	7	+		
2428	Bush-buck	13	—	+		Trypanosomes never seen.
2429	Reed-buck	8	9	+		
2431	„	6	10	+		
2445	„	7	8	+		

From these experiments it is shown that antelope may be readily infected with Sleeping Sickness by the bites of artificially-infected tsetse flies. Eleven antelope were used, and in every case a positive result was obtained. It will be remembered that in similar experiments made with cattle the same result was obtained.

2. *If Antelope can be Infected with the Virus of Sleeping Sickness, can they Transmit the Infection to Clean Laboratory-bred Glossina palpalis when these Tsetse Flies are allowed to Feed upon Them? Further, if these Glossina palpalis become infected, can they transmit the Virus to Susceptible Animals?*

It has now been proved that water-buck, reed-buck, and bush-buck can be infected with the virus of Sleeping Sickness, with what would seem to be unfailing regularity. Should, however, these antelope be incapable of infecting the *Glossina palpalis* with *Trypanosoma gambiense*, the fact is of academic importance only. On the other hand, should the results of feeding clean laboratory-bred *Glossina palpalis* on these infected buck give positive results when these flies are subsequently fed on normal susceptible animals, a further step has been made in the search for a reservoir of the virus of Sleeping Sickness, other than man and his domestic animals.

The method adopted to test this second query was carried out as follows:— Clean, laboratory-bred *Glossina palpalis* were fed for several days on an infected buck. After an interval of starvation of 24 hours or more the flies were transferred to healthy animals and fed daily. When the healthy animal showed *Trypanosoma gambiense* in its blood the experiment was stopped, and the surviving flies were dissected as soon as possible.

The result of 24 experiments carried out on these lines is given in the following table :—

Table II.—Giving the Result of Feeding Laboratory-Bred Flies on Antelope Infected with Sleeping Sickness.

No. of experiment.	No. of clean flies used.	Species of antelope flies fed on.	No. of days flies fed on antelope.	No. of days before flies became infective.	Result.		Remarks.
					Positive.	Negative.	
2346	160	Bush-buck 2328	12	29	+		Buck 2328 never showed *T. gambiense* in blood. In spite of this, flies fed on it became infected 55 days after the buck's infection.
2384	100	,,	8	28	+		
2414	70	,,	6	29	+		
2501	100	,,	8	39	+		
2351	100	Reed-buck 2357	7	41	+		Buck 2357 showed *T. gambiense* in its blood for 5 days only.
2500	100	,,	8	—		−	
2510	100	,,	5	—		−	
2507	200	Reed-buck 2359	6	44	+		Buck 2359 showed *T. gambiense* in its blood for 7 days only.
2421	50	Bush-buck 2371	6	—		−	Buck 2371 showed *T. gambiense* in its blood for 3 days only.
2477	60	,,	6	29	+		
2499	100	Bush-buck 2372	8	—		−	Buck 2372 showed *T. gambiense* in its blood for 2 days only.
2451	95	Water-buck 2378	6	30	+		Buck 2378 never showed *T. gambiense* in its blood.
2478	60	,,	6	—		−	
2559	50	,,	4	—		−	
2454	110	Reed-buck 2427	6	24	+		Buck 2427 showed *T. gambiense* in its blood for 4 days only.
2456	60	,,	4	33	+		
2508	100	,,	6	30	+		
2485	50	Bush-buck 2428	7	28	+		Buck 2428 never showed *T. gambiense* in its blood.
2460	50	Reed-buck 2429	4	27	+		Buck 2429 showed *T. gambiense* in its blood for 3 days only.
2543	100	,,	6	49	+		
2464	55	Reed-buck 2431	3	28	+		Buck 2431 showed *T. gambiense* in its blood for 4 days only. In spite of this, flies fed on it became infected 81 days after its infection.
2544	90	,,	6	36	+		
2592	100	,,	5	43	+		
2476	50	Reed-buck 2445	4	—		−	Buck 2445 showed *T. gambiense* in its blood for 6 days only.

On glancing at Table II it will be seen that 24 experiments in all were carried out. Of these, 17 were positive (70·84 per cent.), and seven negative (29·16 per cent.). The shortest time which elapsed before the flies became infective was 24 days, the longest 49 days, and the average 33·35 days.

Compare these results with those detailed in the 'Proceedings' of the Royal Society, B, 1910, vol. 82, p. 374, Table III. Of the 42 experiments there described, only 8 (19 per cent.) were positive. The clean laboratory-bred flies were fed on *Trypanosoma gambiense*-infected monkeys in 36 of those experiments, in one case on a Sleeping Sickness patient, and in five cases on oxen infected with the virus of Sleeping Sickness.

Positive results were obtained from all the buck on at least one occasion, with the exception of bush-buck, Experiment 2372, and reed-buck, Experiment 2445. Only two experiments were carried out from these buck, one from each, viz., Experiments 2499 and 2476. In Experiment 2499 the flies were fed on the antelope 19 days after the trypanosomes had disappeared from its blood, as far as microscopical examination went. In Experiment 2476 the flies were non-infective to monkeys up to the 45th day after their first feed on the infected buck. This latter experiment was proceeding when the Commission left Uganda, and a positive result may yet have to be recorded.

The most significant of the above observations is the one in which it is shown that 55 days after the last feed of infected *Glossina palpalis* on bush-buck, Experiment 2328, the blood of this buck was capable of infecting clean laboratory-bred flies, though *Trypanosoma gambiense* had never been seen in its blood.

To illustrate how these experiments were carried out, full details of two are given. They are typical of the methods adopted. One positive and one negative experiment have been chosen. (See next page.)

These experiments show that antelope of the water-buck, reed-buck, and bush-buck species, when infected with the virus of Sleeping Sickness, can transmit the infection to clean laboratory-bred *Glossina palpalis*. The infected antelope's blood was, in one case, infective to *Glossina palpalis* for at least 81 days, and in another for at least 55 days. These experiments further show that the flies, when infected by the virus of Sleeping Sickness obtained from the blood of infected antelope, are capable of transmitting the virus to susceptible animals.

Experiment 2501.—To ascertain if Laboratory-Bred *Glossina palpalis* become Infective when Fed on Antelope whose Blood contains *Trypanosoma gambiense.*

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910.				
May 23—28 ...	1—5	Flies fed 5 minutes daily on Bush-buck 2328.		100 flies used.
„ 29	6	Flies starved.		
„ 30—31 ...	7—8	Flies again fed 5 minutes daily on Buck 2328.		
June 1.....	9	Flies starved.		
„ 2—July 7	10—45	Flies fed 5 minutes daily on normal Monkey 2517.		July 8, Monkey 2517 shows *T. gambiense* in its
July 8	46	Flies starved, as Monkey 2517 shows *T. gambiense* in its blood.	+	blood to-day. Allowing 7 days for incubation of *T. gambiense* in monkey's
„ 9	47	The 57 surviving *G. palpalis* dissected; 20·3 per cent. of these flies showed heavy intestinal infection with flagellates (*T. gambiense*).		blood, then the *G. palpalis* became infective on the 39th day after their first infected feed on Buck 2328.

Remarks.—This is the experiment referred to above, where the blood of a buck was capable of infecting clean laboratory-bred flies 55 days after last feed of infected flies on the buck.

Experiment 2499.—To ascertain if Laboratory-Bred *Glossina palpalis* become Infective when Fed on Antelope whose Blood contains *Trypanosoma gambiense.*

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910.				
May 23—28	1—5	Flies fed 5 minutes daily on Bush-buck 2372.		100 flies used.
„ 29	6	Flies starved.		
„ 30—31	7—8	Flies again fed 5 minutes daily on Buck 2372.		
June 1	9	Flies starved.		Monkey 2552 was exam-
„ 2—11	10—19	Flies fed on Cock 2518.		ined bi-weekly from
„ 12	20	Flies starved.		June 13 to August 12.
„ 13—July 12	21—50	Flies fed 5 minutes daily on clean Monkey 2552.	−	It remained healthy.
July 13 and following days.	51	Flies allowed to die. Not dissected as experiment negative.		The surviving *G. palpalis* were not dissected as experiment was negative.

3. *If Glossina palpalis can be infected with the Virus of Sleeping Sickness by Feeding on the Blood of Trypanosoma gambiense-infected Antelope, what Percentage are found to be so Infected?*

It has been shown now that antelope can be infected with the virus of Sleeping Sickness, that when so infected they can infect the fly, and the fly in its turn can convey the disease to susceptible animals.

These facts form a serious sequence of events, which constitute a danger not formerly appreciated in the administrative measures adopted to check the spread of the disease. What is the extent of the danger? A part of this large and important query can be answered if one can give an idea of the percentage of *Glossina palpalis* that become infected with the virus of Sleeping Sickness after they have fed on the infected antelope.

Throughout all these experiments only clean laboratory-bred flies were employed. The fact that there is no hereditary transmission of trypanosomes in *Glossina palpalis* is considered to have been so conclusively proved that two of the members of the Commission have allowed several hundreds of clean laboratory-bred flies to bite them. Further, no evidence has ever been obtained by the Commission that these flies became infected with any flagellate by contact with other flies or fouled cages. Thus, any flagellates found in the laboratory-bred *Glossina palpalis* in these experiments must be considered to be derived from the infected antelope.

In some of the experiments the flies were fed, for varying periods, upon fowls. As will be shown in a further paper, the Commission found an avian trypanosome in some of the fowls obtained for experimental purposes. It would, therefore, be a fair criticism to state that a percentage of the flagellates found on dissection of the *Glossina* were avian in origin, were it not for the fact that negative experiments went to prove that this fowl trypanosome did not develop in the *Glossina*. It is also true that on one occasion the Commission thought they had succeeded in infecting a fowl with *Trypanosoma gambiense*; it may, therefore, be argued that the fowls fed upon in some of these experiments were naturally infected with *Trypanosoma gambiense*, and that the *Glossina* obtained their infection from such naturally-infected fowls and not from the antelope. Though many experiments were devised and carried out to try and confirm this one positive result, all efforts to infect fowls with Sleeping Sickness were so uniformly negative that the Commission must consider the one "positive" result to be an error.

A reference to Table III will show that *Glossina palpalis* were infected by antelope blood where no fowls were ever fed on; in fact, it will be noticed

Table III.—Giving the Percentage of Flies which became Infected when Fed on Infected Antelope. Flies fed at first on the infected antelope and afterwards only on healthy monkeys.

No. of experiment.	Species of antelope flies fed on.	No. of flies used.	No. of flies dissected.	No. of infected flies found.	Percentage of infected flies.	Remarks.
2346	Bush-buck 2328	160	122	21	17·2	The gut-contents of 6 infected flies injected into a rat gave it Sleeping Sickness.
2384	„ 2328	100	91	10	11·0	No injection of infected flies.
2414	„ 2328	70	70	9	12·8	Five infected flies injected into a rat gave it Sleeping Sickness.
2501	„ 2328	100	57	12	21·0	No injection of infected flies.
2351	Reed-buck 2357	100	84	9	10·7	„ „ „
2507	„ 2359	200	80	2	2·5	„ „ „
2477	Bush-buck 2371	60	47	4	8·5	„ „ „
2478	Water-buck 2378	60	—	—	—	Negative experiment. Flies not dissected.
2559	„ 2378	60	—	—	—	„ „ „
2454	Reed-buck 2427	110	92	13	14·2	One infected fly injected into a rat gave it Sleeping Sickness.
2485	Bush-buck 2428	50	26	3	11·5	No injection of infected flies.
2460	Reed-buck 2429	50	38	5	13·1	„ „ „
2543	„ 2429	100	—	—	—	Negative experiment. Flies not dissected.
2544	„ 2431	90	53	5	16·6	No injection of infected flies.
2592	„ 2431	100	78	1	1·3	„ „ „
2476	„ 2445	50	—	—	—	Negative experiment. Flies not dissected.

Remarks.—Of these 16 experiments, 12 were positive and 4 negative. In the positive experiments, 838 flies were dissected. Of these, 94 showed heavy intestinal infection with developmental forms of *Trypanosoma gambiense*, viz., 11·2 per cent. Of these infected flies, 43 were males and 51 females. If to these 838 flies be added all the *Glossina palpalis* of the negative experiments, we get a total of 1108 flies, and a percentage of 8·5 infected. In only 3 of the positive experiments was an injection made into susceptible animals of the citrated gut-contents of the infected flies. All three gave positive results.

that a higher percentage of negative results were obtained where fowls were introduced into the experiments. In some of the experiments the flies were dissected as they died throughout the whole experiment, and in others the flies were dissected only when the experiment became positive. If the experiment was a negative one the flies were not dissected. In order to avoid over estimations of the percentage of infected flies, no fly was called infected unless its gut was *swarming* with trypanosomes, and all the flies dissected during the earlier days of the experiments have been included.

No fly was found to be infected with trypanosomes before the 19th day after its first infected feed on a buck. The tables otherwise explain themselves.

The methods of procedure adopted in the experiments detailed in the following table were precisely the same as those of Table III, with this one exception: The flies were, for varying periods, fed upon fowls as well as upon monkeys.

Table IV.—Giving the Percentage of Flies which became Infected when Fed on Infected Antelope. Flies fed at first on the infected antelope and afterwards on fowls, before being fed on healthy monkeys.

No. of experiment.	Species of antelope flies fed on.	No. of flies used.	No. of flies dissected.	No. of infected flies found.	Percentage of infected flies.	Remarks.
2500	Reed-buck 2357	100	—	—	—	Negative experiment. Flies not dissected.
2510	„ 2357	100	—	—	—	„ „
2421	Bush-buck 2371	50	—	—	—	„ „
2499	„ 2372	100	—	—	—	„ „
2451	Water-buck 2378	95	93	2	2·1	No injection of infected flies.
2456	Reed-buck 2427	60	50	9	18·0	„ „
2508	„ 2427	100	68	6	8·8	Three infected flies injected into a rat gave it Sleeping Sickness.
2464	„ 2431	55	53	8	15·0	No injection of infected flies.

Remarks.—It will be seen that when the flies were fed on fowls and monkeys in these eight experiments, four were positive and four negative. In the positive experiments 264 flies survived for dissection: of these 25 showed heavy intestinal infection with developmental forms of *Trypanosoma gambiense, i.e.* 9·47 per cent. Of the infected flies 11 were males and 14 females. If to these 264 flies be added all the *Glossina palpalis* of the four negative experiments, we get a total of 614 flies and a percentage of 4·0 infected. In the only experiment where an injection was made into a susceptible animal of the pooled citrated gut-contents of infected flies, the result was positive.

An analysis of Tables III and IV brings out the following interesting points:—

In Experiment 2501, Table III, it is seen that 21 per cent. of the *Glossina palpalis* were infected, out of the 57 flies that survived for dissection on the 47th day of the experiment. These flies were infected by bush-buck, Experiment 2328, which had never shown *Trypanosoma gambiense* in its blood; and 55 days had elapsed since any infected *Glossina* had fed on this buck.

Sixteen out of the twenty-four experiments were positive. If all the *Glossina palpalis* dissected in these positive experiments be grouped

together, it is seen that a total of 1,102 flies were examined. Of these, 119 flies—54 male and 65 female—were infected with developmental forms of *Trypanosoma gambiense*—that is, 10·8 flies in every hundred became infected, the sexes being about equally implicated. The highest percentage of infected flies in any one of the positive experiments was 21 per cent., in Experiment 2501, and the lowest was 1·3 per cent., in Experiment 2592. If to the total of 1,102 flies dissected in the positive experiments be added *all* the *Glossina* used in *all* the negative experiments, we get a total of 1,722 flies employed, and 6·9 flies in every hundred infected.

It is perhaps worth noting that a diet for the fly of antelope, fowl, and monkey blood gave a higher percentage of *negative* results and a lower percentage of flies infected than a diet of antelope and monkey blood only. This was quite unexpected, for the Commission, as a result of many experiments and considerable experience, were of the opinion that fowl's blood assisted the development of *Trypanosoma gambiense* in *Glossina palpalis*.

4. *How does Sleeping Sickness Affect the Health of Antelope?*

This point is of considerable importance. If the disease killed the antelope within a short time of infection, or even if it seriously affected their health so as to render them incapable or unwilling to move about freely, the facts detailed above would lose some part of their practical value.

The word "health" is not used here in a technical sense—that is to say, the health was not estimated by a series of blood counts and temperature charts. Interesting as such observations would have been, the Commission regret they were too short-handed and too much pressed by other work to carry them out.

Careful observations were made daily to answer the following questions:—Did the infected antelope during the time they were under observation appear sick? Did they become emaciated? Was there loss of health and strength? Were there corneal opacities, œdematous swellings, conjunctival discharges, or staring coats? These questions may at once be answered in the negative, except in the case of Reed-buck 2445, which will be referred to later below.

When the antelope were brought to the laboratory by the native hunters they invariably suffered from exhaustion, due probably to a combination of causes, such as fright, confinement for two to four days in cages too small to allow free movement, insufficient water and food, and the rough usage undergone when being caught. As a result of these unfavourable con-

ditions, each antelope was kept under observation for a week or two before any experiments were undertaken. During this time they were well fed and comfortably housed in reed kraals erected in a fly-proof house. Some of the antelope, especially the "oribi" and "entalaganya," died during the first fortnight. The 11 survivors were the subjects of these experiments.

Nine of these buck were kept under daily observation for four months after becoming infected with *Trypanosoma gambiense.* They remained apparently in perfect health.

The remaining two antelope were reed-buck. One, Experiment 2429, lived, and appeared very healthy, for 93 days after its infection. It then accidentally broke its leg and had to be killed. A *post-mortem* examination was made, and no evidence of trypanosomiasis was found. The other buck, Experiment 2445, arrived at the laboratory in a poor state of health, and died 12 days after its infection. There was no sign of trypanosomiasis at the *post-mortem* examination.

It is therefore evident that antelope infected with the virus of Sleeping Sickness may live in apparently perfect health for at least four months, and this, though they be kept under conditions less favourable than would occur in nature, the constant handling and fly feedings to which these buck were subject being borne in mind.

5. *Are Antelope Living in the Fly-Area Naturally Infected with Sleeping Sickness?*

Positive evidence on the last query would complete the chain of evidence that antelope living in the fly-areas may act as a reservoir of the virus of Sleeping Sickness. So far it has only been proved that they are "potential" hosts.

The only method by which this query can be investigated is by capturing game in the fly-area and then—

1. Injecting its blood into animals susceptible to *Trypanosoma gambiense* infection.

2. Feeding cages of clean laboratory-bred *Glossina palpalis* on the newly-killed buck and subsequently endeavouring to infect animals susceptible to *Trypanosoma gambiense* with these flies. (The Commission know by observation that *Glossina palpalis* will feed readily on animals newly killed.)

Those who know the local conditions will appreciate the difficulty of carrying out these methods of investigation. It would be necessary for large drives of the buck to be organised in the fly-areas. Several hundred

natives would be required. Large numbers of clean laboratory-bred *Glossina* must be available, many normal susceptible animals must be in readiness, and, at the same time, be so situated that they cannot be bitten by the possibly infected wild flies in the neighbourhood. The laboratory work in hand may have to be abandoned for the time being, and some 50 or 60 buck must be captured or killed before reliable information is forthcoming. The sun is hot, the country very difficult, and the exposure to the bites of the fly very great.

It may be said at once that the Commission were only able to shoot two buck on the Lake-shore. The blood of these gave negative results for *Trypanosoma gambiense* when injected into susceptible animals. One of them, however, gave a positive result for *Trypanosoma vivax*. This trypanosome was proved by the Commission* to be not uncommonly carried by wild Lake-shore *Glossina palpalis*. It is therefore not unreasonable to suppose that at least one of the buck shot had been fed on by the Lake-shore fly.

It was recognised at length that occasional week-end shoots by a member of the Commission were quite inadequate. The assistance of the Acting-Governor of Uganda, S. C. Tomkins, Esq., C.M.G., the Acting Principal Medical Officer, Dr. C. Wiggins, of the Uganda Medical Staff, the Provincial Commissioners of Kampala, F. A. Knowles, Esq., C.M.G., and L. H. Cubitt, Esq., and of Sir Apolo Kagwa, K.C.M.G., the Prime Minister, was then sought to aid in organising a series of large drives of wild game in the fly-area in the neighbourhood of the laboratory. We are greatly indebted to these gentlemen for their ready efforts on our behalf, which resulted in a large drive being organised. Unfortunately, the Commission were ordered to leave the country before the drive could take place. The work on these lines, however, is being continued by Dr. R. van Someren, of the Uganda Medical Staff, and Captain A. D. Fraser, Royal Army Medical Corps, who were instructed to take over the laboratory work.

This very difficult question, therefore, still awaits its answer.

Conclusions.

1. Water-buck, bush-buck, and reed-buck can readily be infected with a *human strain* of the trypanosome of Sleeping Sickness by the bites of infected *Glossina palpalis*.

2. One exposure to the bites of infected flies is sufficient to infect an antelope with the virus of Sleeping Sickness.

3. Though the blood of an antelope may be proved to be infected with

* *Vide* 'Roy. Soc. Proc.,' B, 1910, vol. 82, pp. 63—66.

× See page 313. Evidence seems hardly sufficient.

Trypanosoma gambiense, careful and continued examinations over prolonged periods may fail to reveal the presence of the parasite in the blood.

4. The incubation of the disease (Sleeping Sickness) in antelope is probably seven days.

5. Antelope of the water-buck, bush-buck, and reed-buck species, when infected with the virus of Sleeping Sickness, can transmit the infection to clean laboratory-bred *Glossina palpalis*.

6. This transmission of the infection to clean laboratory-bred flies may occur at least 81 days after the last feed of the infected flies on a buck.

7. *Glossina palpalis*, when infected with the virus of Sleeping Sickness obtained from the blood of infected antelope, are capable of transmitting the virus to susceptible animals.

8. An appreciable percentage of *Glossina palpalis* will become infected with the virus of Sleeping Sickness should these flies feed on antelope suffering from this disease.

9. It follows, from the above conclusions, that antelope living in the fly-areas are "potential" reservoirs of the virus of Sleeping Sickness.

10. No antelope up to the present has been found naturally infected with *Trypanosoma gambiense*.

Harrison and Sons, Printers in Ordinary to His Majesty, St. Martin's Lane.

WELLCOME LIBRARY INSTITUTE